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# Degradation of tannin at high concentrations by a bacterial consortium isolated from tannery effluent soil

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## ABSTRACT

Tannins are defined as naturally occurring water-soluble poly-phenolic compounds having wide prevalence in plants. Hydrolysable and condensed tannins are the two major classes of tannins. These compounds have a range of effects on various organisms, from toxic effects of animals to growth inhibition of microorganisms. Catechin a major component of tannin is considered to be recalcitrant and some microorganisms have developed mechanisms to degrade catechin. Tannase, a key enzyme in the degradation of hydrolysable tannins, is present in a diverse group of microorganisms, including rumen bacteria. This enzyme is being increasingly used in a number of processes. Presently, there is a need for increased understanding of the biodegradation of condensed tannins. The aim of this study was to enrich and isolate bacterial consortium from a tannery soil that are capable of utilizing higher concentration of tannin and catechin as sole source of carbon. We have assessed the aerobic biodegradation of catechin and tannin by the consortium that was isolated from a polluted soil by enrichment with tannin. The results of the experiments demonstrated that the consortium could degrade 500mg/L of tannin and 750mg/L of catechin, where complete degradation was achieved in 96 h. The mixtures of tannin and catechin gave a removal efficiency of 79 % and 87 % respectively. Isolation of such consortium might be useful for the treatment of industrial wastes particularly in environments contaminated with tannery wastewater. © 2010 Trade Science Inc. - INDIA

## KEYWORDS

Biodegradation;
Tannin;
Catechin;
Poly-phenols;
Tannery wastewater.

## INTRODUCTION

Tannins are defined as naturally occurring water-soluble polyphenols of varying molecular weight, which differ from most other natural phenolic compounds in their ability to precipitate proteins from solutions[1]. This property is the basis for their past and present use in the tanning industry. The presence of tannins in the wastewaters would cause many problems associated with environmental pollution and wastewater treatment. Owing to the presence of tannins, the wastewaters are usually highly colored, which is very difficult to be eliminated by common methods like dilution and adsorption[2]. In addition, tannins can inhibit growth of micro-

organisms and therefore, are toxic to activated sludge. This negative effect can be observed when tannin-containing wastewaters are biologically treated in wastewater treatment plants[2,3]. Based on their structures and properties, they are distributed into two major groups-hydrolysable and condensed tannins. Hydrolysable tannins are composed of esters of gallic acid (gallotannins) or ellagic acid (ellagitannins) with a sugar core which is usually glucose, and are readily hydrolysed by acids or enzymes into monomeric products. Condensed tannins, also known as polymeric proanthocyanidins, are composed of flavonoid units, and are usually more abundant in tree barks and woods than their hydrolysable counterparts. Catechin is a major component of the condensed tannin, considered to be recalcitrant. Catechins and phenols are the major organic pollutants of tannery effluent and impart brown colour to receiving waters[5]. Due to the recalcitrant nature, catechins are highly resistant to microbial attack and cause pollution to the environment.

Tannins inhibit the growth of a number of microorganisms, resist microbial attack and are recalcitrant to biodegradation[6]. Condensed tannins are more resistant to microbial attack than hydrolysable tannins and are toxic to a variety of microorganisms. As mentioned above, tannins are able to inhibit growth of microorganisms in general. But it has been found that some microbes are resistant to tannins and can grow with tannins as carbon and energy source[2]. Tannins are utilized by many fungi, bacteria and yeasts are quite resistant to tannins, and can grow and develop on them[7]. Certain moulds such as *Aspergillus* or *Penicillium* have been observed to grow on the surface of liquids of tannery pits and tannery wastes[8]. Tannin degradation has been reported by many researchers[9-13]. Recently tannin degradation has been reported in vegetable tanning wastewater where the wastewater was diluted and the removal extent of COD and tannins were more than 50 %[14]. Catechin the group that occupies an intermediary position in the tannin hierarchy is reported to be degraded by both bacteria and fungi[15-21]. *Sporotrichum pulverulentum* was used for fermentation of oak leaves where the condensed tannins were reduced in 10 days[22]. The purpose of this work was to investigate degradation of higher concentration of tannin and catechin by a bacterial consortium isolated from tannery contaminated site in Chennai.

## MATERIALS AND METHODS

### Enrichment and isolation of the bacterial consortium

Soil samples were collected and screened from different sites of tannery contaminated soils (Tannery industry, Pallavaram, Chennai). A standard enrichment method was used for isolation, the substrates were provided as sole carbon and energy source in the mineral medium containing (in g/L) 1.0- $KH_2PO_4$, 3.0-$NH_4PO_4$, 8.0-$NH_4SO_4$ ,1.0- $MgCl_2$, 20- Agar, pH - 6.8.[23]. The medium was autoclaved, cooled to room temperature and was amended with wattle tannin (100mg/L) through a sterile filter (0.45 μm) in 250ml Erlenmeyer flasks. After acclimatization of the cultures, the culture was transferred several times after every four days. Increase in cell count from $10^4$ to $10^9$ cfu/mL on tannin as sole carbon source was taken as a confirmation of the ability of the consortium to utilization of tannin. All the experiments were conducted in duplicates. The consortium was maintained on mineral salts medium with tannin as the sole carbon and energy source. During all these experiments, after observing the turbidity at an interval of 24h, 5ml aliquots was aseptically inoculated into 100ml of liquid medium supplemented with increasing tannin concentrations. The chemicals and reagents used in the study were analytical grade.

### Biodegradation on catechin and tannin

Growth of the bacterial consortium containing bacterial strains was studied by analyzing the colony forming units (CFUs) per ml by dilution and plate count on agar medium (MSM with tannin and 1.5% agar) and pH was adjusted to 7. After growth at 100mg/L of tannin, when the cells concentration reached 0.5 (OD600), an aliquot of the culture was centrifuged at10,000 rpm and 4°C for 15 min. To clean the biomass, it was resuspended in the phosphate buffer and centrifuged. The cells were inoculated into 100ml of mineral salts medium supplemented with increasing concentration of tannin and catechin respectively. Tannin was estimated spectrophotometrically by ammonium molybdate

FULL PAPER

method and catechin was estimated by ferrous tartarate method (Sambhandam 1983). The concentrations of tannin used were 100mg/L to 500 mg/L and catechin was at concentrations, 250mg/L, 500mg/L and 750mg/L.

## RESULTS AND DISCUSSION

### Degradation of tannin

Soil samples were collected from tannery affected soils to isolate tannin degrading bacterial strains. Several bacterial strains were isolated which could degrade tannin compounds. Initially, during the isolation period several bacterial strains were viable after repeated transfers in the adaptation phase only few gram negative rods prevailed as a consortium in utilizing tannins as the sole carbon source. This condition could be due to selection within the population for specific bacterial strains which are more tolerant to the substrate, probably due to changes in the regulatory system, achieved by substrate specificity or over expression of a pre-existing system for degradation of related substrates[24].

It is well known that tannins are toxic and bacteriostatic compounds making non-reversible reactions with proteins[25]. Nevertheless, some bacteria may degrade many phenolic compounds including natural ones like catechol and protocatechuic acid[6,7]. In previous studies, degradation of tannin has been reported by individual strains of bacteria, yeasts and fungi under aerobic conditions[6,7,26] and consortium was used under anaerobic conditions in the degradation of gallotannins[6] who observed the breakdown of tannic acid. Deschamps et al. (1980) made a detailed study on this phenomenon and isolated fifteen bacterial strains belonging to the genera *Bacillus*, *Staphylococcus*, and *Klebsiella* by enrichment culture technique, using tannic acid as the sole source of carbon Nine of the isolated strains grew both on tannic acid and gallic acid, whereas only four strains degraded catechol or catechin. Most of the fungal species that have been used for biodegradation of tannery effluent, belong to the genera *Aspergillus* and *Penicillium.* Other fungi, including *Chaetomium*, *Fusarium*, *Rhizoctonia*, *Cylindrocarpon*, and *Trichoderma*, are capable of

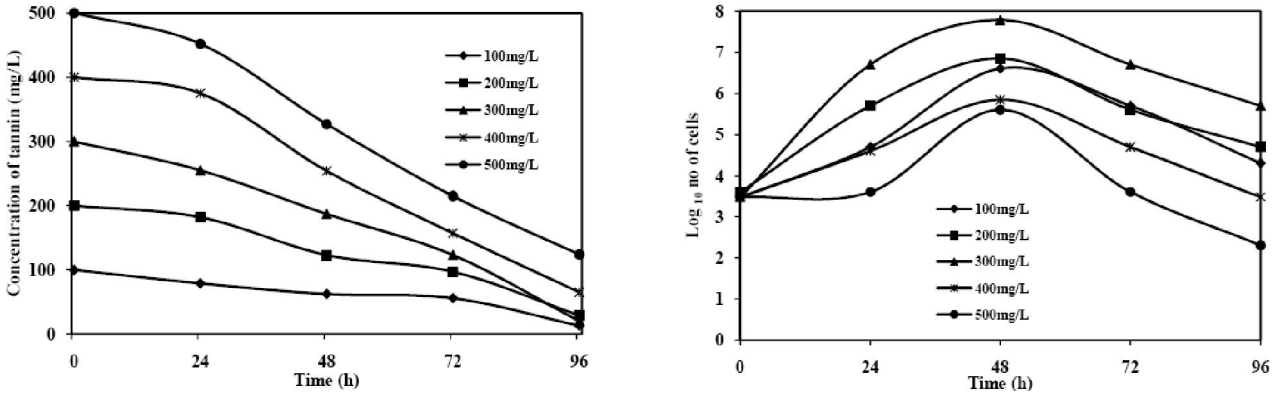


**Figure 1 : Degradation and growth of the bacterial consortium on tannin**

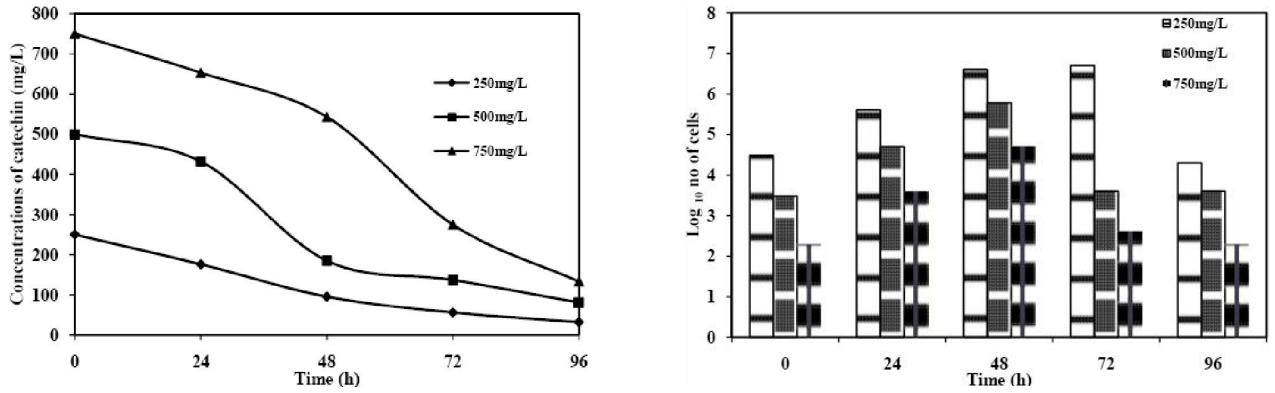


**Figure 2 : Degradation and growth of the bacterial consortium on catechin**

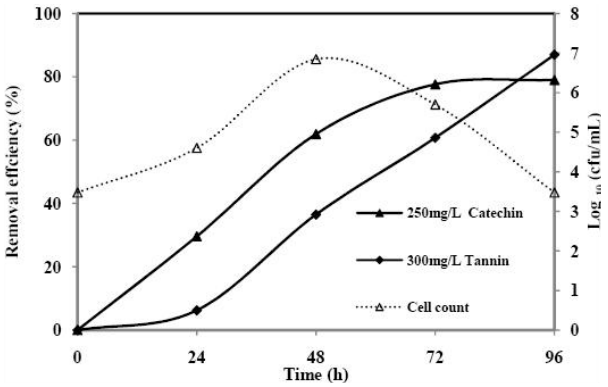


**Figure 3 : Degradation and growth of the bacterial consortium on the mixtures of tannin**

degrading tannery waste constituents[15]. *Psalliata campestris* was found to oxidise catechin and *A. niger* could degrade gallic acid[26].

In present study bacterial consortium was used for the degradation of tannin, catechin and mixtures of both. Batch studies were conducted with tannin concentrations from 100mg/L to 500mg/L. The degradation of tannin with the growth of the consortium is presented in the figure 1. The consortium was able to utilize tannin showing highest degradation efficiency at 300mg/L of tannin, where the removal efficiency was about 93 % with the cell count increasing from $3 \times 10^3$ to $6 \times 10^7$. In the case of lower concentrations of tannin the degradation efficiency was 87 % and 86 % with growth maximum of $4 \times 10^6$, $7 \times 10^6$ at 100mg/L and 200mg/L of tannin concentrations. When the concentration of tannin was increased to 400mgL the removal efficiency reduced to 82 % correlating with decrease in the growth phase with $7 \times 10^5$cfu/ml. The degradation of 500mg/L tannin was relatively toxic than other concentration, here the removal efficiency was lowered to 75% with decrease in cell count in the log phase to $5 \times 10^5$. More recently *Sporotrichum pulverulentum* was used in the fermentation of oak leaves which decreased the contents of total phenols and condensed tannins by 58 and 66% respectively in 10 d. Further increase of fermentation time up to 40 d did not increase the tannin degradation substantially[22].

## Degradation of catechin

Catechin is a group that occupies an intermediary position in the tannin hierarchy as a family of catechin tannins. Batch studies were conducted with the bacterial consortium at different concentrations of catechin starting from 250mg/L, here the removal efficiency was 87% with growth of $7 \times 10^6$ was achieved in 96 h. When the concentrations was increased to 500 mg/L the removal efficiency decreased to 84% with cell count of $6 \times 10^5$, it still reduced at higher concentration of 750mg/L to 82% were the growth in late log phase reached to $5 \times 10^4$. The growth and degradation of the compound is given in the figure 2. Brezillon et al.[27] isolated catechin degrading strains from faeces of HF rats receiving catechin, here 10 mM of the substrate was degraded from 25% to 65% in 7 days. Osawa et al.[28] reported the occurrence of lactobacilli capable of degrading hydrolysable tannins in human gut microflora and foodstuffs were the concentrations of tannins were less. Current work with the bacterial consortium was able to mineralize the compounds in 96 h.

## Degradation of mixtures of tannin and catechin

The presence of tannins in the wastewaters makes it highly coloured, which is very difficult to be eliminated by common methods like dilution and adsorption[2]. Many methods have been explored to treat tannin-containing wastewaters, such as adsorption[29,30], membrane filtration[31,32], photocatalytic and sonochemical degradation[33,34]. But, in practice, tannin containing wastewaters are usually successively treated by chemical settling and biodegradation so as to meet the discharging specifications. Tannin is highly soluble in water, and is present in mixtures of its derivatives in tannery wastewater. To check the ability of the consortium to utilize the mixtures of tannins, this study was conducted with optimized concentrations of catechin and tannin. In batch studies with individual compounds of tannin and catechin, tannin showed optimum degradation with 300mg/L and catechin 250mg/L. The growth of the consortium with mixtures of both the compounds with its removal efficiency is given in the figure 3. The consortium utilized the tannin mixtures, where the growth increased from $3 \times 10^4$ to $6 \times 10^6$, with degradation efficiency of catechin 79% and tannin 87% respectively (Figure 3). This is the first report on degradation of mixtures of catechin and tannin in the batch study. From the results it gives hope for degradation of tannins in the tannery wastewaters.

In future study, the enzymes involved during the degradation of tannins will throw light for further use of this

consortium for the treatment of tannery wastewaters.

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